United States Department of Agriculture

Food Safety and Inspection Service

MLG 20.02

Real-Time PCR Method for Species Identification in Meat and Poultry Products

This method describes the laboratory procedure for performing species identification in meat and poultry products using a real-time PCR assay.

Notice of Change

Description and purpose of change(s):

FSIS is responsible for ensuring accuracy and compliance to species labeling requirements. FSIS has adopted a new diagnostic platform that uses a real-time PCR assay for the detection of contaminating species of interest in meat and poultry samples. DNA extraction is performed to obtain pure genetic material which is analyzed on the bioMérieux GENE-UP® PCR instrument. A list of species identified by FSIS using the real-time PCR method is included in the Introduction section (Table 1).

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Introduction

Meat and poultry samples are analyzed using a real-time PCR assay for the detection of the species of interest using the representative GENE-UP® Animal Species ID test. This technology can detect low level adulterants within food samples by utilizing master mixes to target specific animal species and can be run concurrently, allowing testing of multiple targets in a single run. DNA extraction is performed to obtain pure genetic material, and the DNA is quantified to obtain a normalized concentration. The normalized solution is used with the Species ID tests and analyzed on the bioMérieux GENE-UP® PCR instrument.

An outline of this process is shown in Figure 1. Table 1 lists the species identified by this procedure. It should be noted that this assay works on both cooked and raw meat and relies on the ability to obtain intact DNA from the sample. This test was first adapted and used in FSIS to detect Feline and Canine tissue pursuant to S. 3042, Title XII, subtitle E, section 125 of the 2018 Agriculture Improvement Act making the slaughter of these animals illegal for human consumption. Upon approval of the bill, the information can now be found in 9 CFR part 319 – Definitions and Standards of Identity or Composition (https://www.ecfr.gov/current/title-9/chapter-III/subchapter-A/part-319).

The method described in this guidebook are for use by the FSIS laboratories. FSIS does not specifically endorse any of the mentioned test products and acknowledges that equivalent products may be available for laboratory use.

Figure 1. OUTLINE OF ANALYSIS

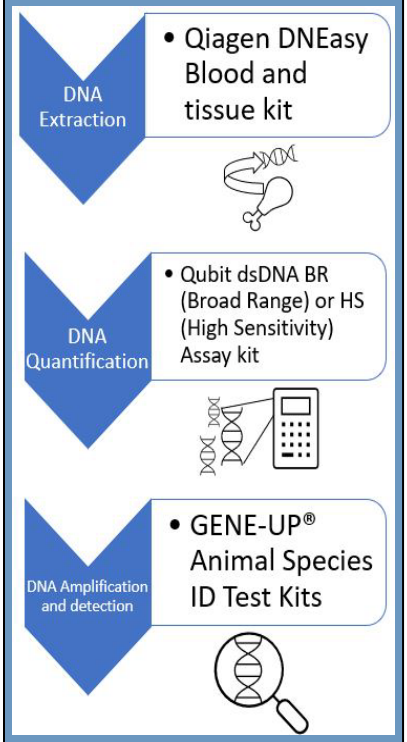

Table 1. Species detected using this method.

Species ID Test Kit Name	Species Detected
Bovine	*Bos taurus, Bison bonasus, B. bison*
Equine	*Equus caballus, E. asinus*
Ovis	*Ovis aries*
Pork	*Sus scrofa, S. scrofa domesticus*
Elk	*Cervus canadensis*
Deer	*Odocoileus virginianus*
Chicken	*Gallus gallus*
Duck	*Cairina moschata, Anas platyrhynchos*
Turkey	*Meleagris gallopavo*
Cat	*Felis catus*
Dog	*Canis lupus*
Rabbit	*Oryctolagus cuniculus*
Goat	*Capra hircus*

For Siluriformes, refer to the Chemistry Laboratory Guidebook method: CLG-FPCR Fish Speciation by PCR/Sequencing of Mitochondrial Cytochrome Oxidase I (COI) Gene.

Safety Precautions

The Safety Data Sheet (SDS) may be obtained from the manufacturer for the media, chemicals, reagents, and microorganisms used in the analysis. The personnel who will handle the material are to read the SDS prior to startup. See "Biosafety Chart" at the end of this chapter for more information.

QUALITY CONTROL

Lab Quality Control Procedures

The species positive DNA control is composed of normalized DNA concentrations of known species tissues that are kept frozen for up to one year. A positive species DNA control and PCR grade water negative control is to be used for each species identification kit used. When not in use, all kit components are to be stored at the appropriate stated temperature to preserve and maintain kit components. Do not mix components from one lot with components from another lot. Observe the manufacturer's expiration date of all test kit components. Kits are not to be used beyond the expiration date.

Equipment, Kits, Reagents and Supplies

Table 2: Equipment and Kits for Species Identification

Equipment	Supplier	Purpose
bioMérieux GENE-UP® PCR instrument	bioMérieux GENE_UP	PCR amplification and detection
Qubit dsDNA Broad Range (BR) Assay kit	Invitrogen, Catalog #Q32850	Quantify DNA concentration
Qubit dsDNA High Sensitivity (HS) Assay Kit	Invitrogen, Catalog #Q32851	Quantify DNA concentration
Qubit Fluorometer	Invitrogen, Catalog #QC33216	Quantify DNA concentration
Water bath or incubator, calibrated to 56 ± 1 °C	General lab supplier	DNA extraction
Microcentrifuge with 1.5 mL tube rotor	General lab supplier	DNA extraction
GENE-UP® Animal Species ID Test Kits	bioMérieux, Catalog #	PCR amplification and detection
Analytical balance, sensitivity to at least ± 0.1 g	General lab supplier	Weighing of sample, pipette verification
Qiagen DNeasy Blood and Tissue Kit	Qiagen, Catalog #69504	Extraction and isolation of genomic DNA
Vortex Mixer	General lab supplier	Mix reagents in tubes
Bead mill tissue disruptor (optional)	General lab supplier	Grind and homogenize sample

Table 3: Reagents and Supplies

Reagent	Supplier	Purpose
95-100% Ethanol	General lab supplier	DNA extraction
De-ionized ultrapure or PCR Grade water (ddH_2O)	General lab supplier	PCR amplification
Sterile, disposable spoons, scissors, forceps, and knives	General lab supplier	Sample preparation
Control species tissue		Experimental control
1.5 mL centrifuge tubes	General lab supplier	Extraction and storage of DNA
1000 µL, 200 µL and 20 µL pipettors with tips	General lab supplier	Add and mixing of reagents
0.5 mL PCR tubes	General lab supplier	Quantify DNA concentration
Small weigh boats	General lab supplier	Weighing sample

Method

Sample Preparation

All samples are prepared as follows:

a. Weigh out and collect 30 ± 5 mg of muscle tissue into a labelled centrifuge tube. A representative sample of the present tissue types is to be taken. Avoid fat and connective tissue.
b. Cut sample into small pieces to facilitate DNA extraction. Alternatively, a bead mill may be used.
c. If necessary, samples may be held frozen (-20 °C or below) at this point before use.

DNA Extraction

Extract DNA from prepared sample using commercially available kits and follow manufacturer's instructions. DNA is eluted in 50-200 µL of elution buffer.

DNA Normalization

a. Quantify purified DNA after extraction using the Qubit dsDNA BR (Broad Range) or HS (High Sensitivity) Assay Kit and follow manufacturer's instructions. DNA concentration is normalized to a 5-10 ng/µL concentration for most samples. When analyzing a pork sample, a minimum concentration of 10 ng/µL is needed to detect 0.2% of pork DNA.
b. Adjust DNA concentration as needed by dilution using molecular grade water or elution buffer from the extraction kit.

PCR Amplification and Detection

a. Follow manufacturer's instructions for use of GENE-UP® instrument and kits.
b. Prepare the needed number of PCR tubes for each species test and place in the PCR tube holder. Promptly return unused tubes to the freezer.
c. A positive species DNA control is to be used for each species identification kit used. Any sample that is found to be non-compliant is to be re-extracted and tested to verify the non-compliant result.

Biosafety Chart

Safety Information and Precautions

1. Required protective equipment: Nitrile or latex gloves, lab coat, and safety glasses
2. Hazards

Procedure Step	Hazard	Recommended Safety Procedures
PCR Amplification and Detection	GENE-UP® equipment may also be used for detection of bacterial food pathogens and appropriate precautions should be taken.	Clean and decontaminate equipment. Follow CDC guidelines for manipulating Biosafety Level 2 (BSL-2) pathogens.

References

A high incidence of species substitution and mislabeling detected in meat products sold in South Africa (2013). Cawthorn D.-M., Steinman H.A. and Hoffman L.C. Food Control, 32, 440-449.

Food Forensics: Using DNA-Based Technology for the Detection of Animal Species in Meat Products (2014). Yosef T.A., Al-Julaifi M.Z. and AL-Rizqi A.M. Nature and Science, 12(6), 82-90.

Contact Information and Inquiries

Inquiries about methods can be submitted through the USDA website via the "Ask USDA" portal at https://ask.usda.gov or please contact:

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This method has been validated, reviewed, approved, and deemed suitable and fit for purpose for use in the USDA FSIS Field Service Laboratories.

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